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Research Article

Isolation of Frankia from Root Nodules of *Casuarina junghuhniana* Using the Liquid Qmod Media

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Abstract

Frankia is a Gram-positive, nitrogen-fixing actinobacterium that forms a symbiotic association with actinorhizal plants, contributing to nitrogen fixation in the soil. It is also found free-living in soil environments. This study investigates the isolation of *Frankia* strains from the root nodules of *Casuarina junghuhniana*, a species that thrives in sodic soils and is known for its ability to withstand environmental stresses such as drought, salinity, and heavy metal contamination. Root nodules were surface-sterilized, processed, and incubated in Liquid Qmod media, which proved to be the most effective medium for *Frankia* growth. The study focused on the morphological characteristics of *Frankia* in culture, such as hyphal outgrowth, sporangia formation, and vesicle production, which are essential for nitrogen fixation. Hyphal outgrowth from vesicle clusters was observed within 3–10 days of inoculation, and the colonies were macroscopically visible within 14–20 days. The formation of sporangia occurred after 7–30 days, depending on the medium composition. Vesicles formed in nitrogen-deficient media were critical sites of nitrogen fixation, promoting plant growth. The results affirm that *Frankia* plays a key role in enhancing the growth and stress tolerance of *Casuarina* species, and highlight the effectiveness of Qmod medium for isolating Frankia from nodules. This study contributes to the understanding of *Frankia*-plant symbiosis and its potential for improving plant productivity in challenging environments.

Keywords: *Casuarina junghuhniana;* QMOD Media; Frankia; Isolation; Root Nodules

Introduction

Frankia is slow-growing, Gram-positive, nitrogen-fixing filamentous actinobacterium that forms a symbiotic association with actinorhizal plants, but it can also be found free-living in soil [1]. In the symbiotic association, *Frankia* is an important contributor to nitrogen fixation [2]. Based on the morphology, chemotaxonomy, and 16S rRNA sequences, the genus *Frankia* is assigned to the phylum actinobacteria and order *Frankia*les [3]. *Frankia* has been subjected to genomic studies toward its characterization for better understanding and functional role of actinorhizal symbiosis [4]. *Frankia* has a high GC content and grows rather slowly [5]. In liquid culture, depending on the condition of culture, *Frankia* forms hyphae and sporangia. The latter is located on hyphae terminally [5]. The hyphae are septate, whereas sporangia are multilocular and contain spores that are the agents of asexual propagation. *Frankia* in culture produces vesicles that are lipid-encapsulated roughly

spherical structures, attached to hyphae by short stalks. Vesicles are sites of nitrogenous activity and are generally formed when nitrogen in the medium is deficient [4]. Morphology of *Frankia* in the root nodules varies depending on the actinorhizal species whereas the size of hyphae and presence or absence of vesicles depends on the actinobacterial species [7]. The symbiosis between actinorhizal plants and *Frankia* induces the formation of a perennial root organ called nodule, wherein bacteria are hosted and nitrogen is fixed [8]. In the field, the actinorhizal nodules can be of various forms and colors [2]. Two types of nodule formation occur in actinorhizal symbiosis: the intracellular and intercellular infections [6].

Actinorhizal plants *Casuarina* and *Alnus* are important in view of many applications.

Especially, *Casuarinaceae* trees are widely distributed in tropical countries [9]. This family is composed of 4 genera with 96 species. *Casuarina* spp. can adapt well under stress conditions like drought, flood, salinity, and sites polluted by heavy metals.

Casuarina trees are also used as smokeless fuelwood with high calorific value, as hardwood in the construction of houses in Benin and in the production of paper pulp in India. Association with *Frankia* increases *Casuarina junghuhniana* growth and biomass [6]. Furthermore, in this symbiotic relationship, bacteria confer to plants a high resistance to abiotic and biotic stresses [1].

Materials and Methods

Collection and processing of *Casuarina* root nodules

The *Casuarina junghuhniana* root nodules were collected from sodic soil region which is located in ADAC and RI, Trichy, Tamil Nadu, India. The nodules were washed with sterile distilled water, mixed with clean moist sandy soil in polyethylene bags, and stored at −4 °C in a refrigerator. Fresh nodules were dissected into individual lobes and surface-sterilized for 5–10 min in 10% H_2O_2 and for 15 min in 6% sodium hypochlorite containing a drop of detergent, then washed several times in sterile distilled water.

Media preparation

Basic and important media used in the *Frankia* isolation and further studies related to its growth and activity is Liquid Qmod Media. After the preparation of media, the pH was adjusted to 6.8. Composition of QMOD medium was that described by [10] is as follows.

Figure a

Composition of QMOD media

S. No	Components	Concentration
1	Dipotassium phosphate	0.3 g
2	Monosodium phosphate	0.2 g
3	Magnesium sulphate heptahydrate	0.2 g
4	Potassium chloride	0.2 g
5	Yeast extract	0.5 g
6	Peptone	5.0 g
7	Ferric citrate (Solution)	1 ml
8	Solution oligoelements	1 ml
9	Lecithin	5 mg
10	Distilled water	1 litre
pH adjusted to 6.8		

Table 1

Solution oligoelements

S. No	Components	Concentration
1	Boric acid	1.5 g/l
2	Manganese sulphate heptahydrate	0.8 g/l
3	Zinc sulphate heptahydrate	0.6 g/l
4	Copper sulphate heptahydrate	0.1 g/l
5	Ammonium molybdate tetrahydrate	0.2 g/l
6	Copper sulphate heptahydrate	0.01 g/l

Table 2

Inoculum preparation

Washed in a stream of distilled water to remove loose soil. Root nodules were collected from the *C. junghuhniana* seedling and surface sterilized with 1% HgC12. The root nodules were crushed with sterile water and centrifuged in cooling centrifuge @1000 rpm for 5 minutes. Then the supernatant was collected (1 ml) and made up to 100 ml with sterile water and stored in deep freezer at 4°C. The *Frankia* is the inoculated into the media as described above and observed for its growth and activity.

Results and Discussion

Frankia colony development

Frankia started to grow in the culture media. To ensure that the organisms obtained from the plates were *Frankia* strains, the observation of hyphal outgrowth from vesicle clusters on inverted petri plates by microscopy. Hyphae generally grew from several points on the vesicle cluster. It was not possible to determine if the hyphae originated from vesicles or from internal hyphae in the cluster. Sporangia typical of *Frankia* strains usually developed within 7 days after hyphal emergence on QMOD medium. On plates containing pyruvate or succinate, sporangia were formed only after an extended incubation of 20 to 30 days. Similar findings were found in the study made by [10]. The earliest that hyphal outgrowth was detected microscopically was 3 days after inoculation. Macroscopically visible colonies were observed as early as 14 days after inoculation on FMC with pyruvate as the carbon source. More commonly, *Frankia* strains from nodules obtained during the growing season showed microscopically detectable outgrowth from vesicle clusters within 10 days of inoculation, and colonies were macroscopically visible and ready to be transferred by 20 days after inoculation. *Frankia* vesicles help with growth of *Casuarina* by utilization of nutrients and fixing nitrogen from the atmosphere, facilitating the plant growth [11].

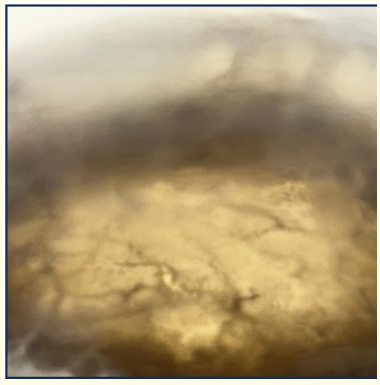

Figure 1: Colony formation by Frankia.

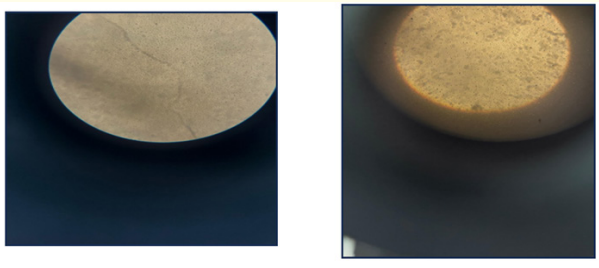

Figure 2: Initial Frankia growth under microscope

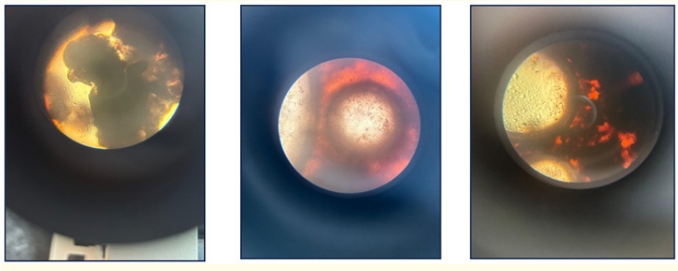

Figure 3: Microscopic observation of Frankia after staining.

Conclusion

From this study it is concluded that the *Frankia* strains have been isolated from the root nodules of the *Casuarina junghuhniana* clone effectively using the best suitable method. It is also confirmed that Qmod media (Liquid Qmod media) is the best suited media for the growth of *Frankia*.

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